HEK-Blue™ IFN-λ Cells

Interferon-lambda reporter cells

Catalog code: hkb-ifnl

http://www.invivogen.com/hek-blue-ifn-l

For research use only

Version 18G03-MM

PRODUCT INFORMATION

Contents:

- **1 vial of HEK-Blue™ IFN-λ cells** (3-7 x 10^6 cells)

IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.

- **1 ml of Normocin™** (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria, and fungi. Store at -20 °C.*
- **1 ml of Blasticidin** (10 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of Puromycin** (10 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of Zeocin™** (100 mg/ml). Store at 4 °C or at -20 °C.*

*The expiry date is specified on the product label.

- **1 ml of QB reagent** and **1 ml of QB buffer** (sufficient to prepare 100 ml of **QUANTI-Blue™ Solution**, a SEAP detection reagent). QB reagent and QB buffer are stable for 1 year at -20 °C. QUANTI-Blue™ Solution is stable for 2 weeks at 4 °C and for 2 months at -20 °C.

Handling Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

*Note: **Avoid freezing cells upon receipt** as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

Cell Line Stability

Cells will undergo genotypic changes over time that will result in reduced responsiveness in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Quality control:

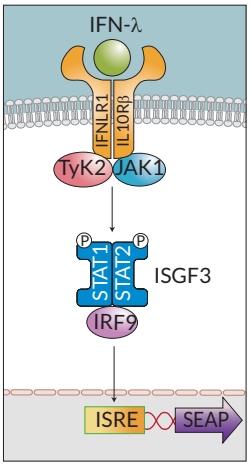
- HEK-Blue™ IFN-λ cells were stimulated by various cytokines. As expected, human and murine IL-28A and IL-28B and human IL-29 induced the production of SEAP. These cells do not respond to type I and type II IFNs (see validation data sheet).
- Biallelic IFNAR2 and IFNGR1 gene knockouts were verified by functional assays, PCR and DNA sequencing.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

INTRODUCTION



The interferon-lambda (IFN-λ) family, also known as type III IFNs, is crucial for antiviral responses at epithelial barriers such as the respiratory and gastrointestinal tracts[1, 2]. In humans, this family comprises four distinct proteins called IFN-λ1 (interleukin-29, IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B), and the poorly secreted IFN-λ4, whose function is not completely understood[1]. In mice two functional orthologs (IL-28A and IL-28B) have been described. IFN-λs are produced when a viral infection is sensed by pattern recognition receptors. Following their production, IFN-λs bind to a heterodimeric receptor (comprising IFNLR1 and IL10Rβ) and recruit the Janus kinases (JAK1 and TyK2). This leads to the phosphorylation of STAT1 and STAT2, which then dimerize and interact with IFN regulatory factor 9 (IRF9), forming the ISGF3 complex. ISGF3 binds to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG) to regulate their expression.

1. Lazear HM. *et al.*, 2015. Interferon-λ: Immune Functions at Barrier Surfaces and Beyond. Immunity. 43(1):15-28. **2. Lee S. & Baldridge MT., 2017.** Interferon-Lambda: A Potent Regulator of Intestinal Viral Infections. Front Immunol. 8:749.

CELL LINE DESCRIPTION

HEK-Blue™ IFN-λ cells were specifically designed to monitor the activation of the JAK/STAT/ISGF3 pathway induced by IFN-λ. They were generated by stable transfection of HEK293 cells with the human IFNLR and IL10R receptor genes, along with the human STAT2 and IRF9 genes to obtain a fully active IFN-λ signaling pathway. These cells are unresponsive to type I and type II IFNs due to IFNAR2 and IFNGR1 gene knockouts.

In order to detect activation of the IFN-λ pathway, HEK-Blue™ IFN-λ cells were further transfected with a reporter gene expressing a secreted embryonic alkaline phosphatase (SEAP), under the control of the ISG54 promoter. ISG54 is a well-known ISG that is activated through an ISRE-dependent mechanism by IFN-λ. Therefore, stimulation with human or murine IFN-λ triggers the JAK/STAT/ISGF3 pathway and induces SEAP production. SEAP can be readily monitored when using QUANTI-Blue™, a SEAP detection medium. These cells respond to human and murine IL-28A and IL-28B, and human IL-29.

These cells are resistant to Blasticidin, Hygromycin, G418, Puromycin, and Zeocin™. They should be maintained in growth medium (as described on the next page) supplemented with Blasticidin, Puromycin, and Zeocin™.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

FAQ Any questions about our cell lines? Visit our FAQ page.

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ IFN-λ cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require **Biosafety level 2** according to CDC guidelines. The biosafety level may vary depending on the country. In the United States, HEK293 cell lines are designated Biosafety Level 2 according to the Center for Disease Control and Prevention (CDC). In Germany, HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

• Growth Medium: DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% fetal bovine serum (FBS), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™

• Test Medium: DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS (30 min at 56°C), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™

• Freezing Medium: DMEM with 20% FBS and 10% (v/v) DMSO

Required Selective Antibiotics

Blasticidin, Puromycin, and Zeocin™

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% (v/v) ethanol.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells in a tube containing 15 ml of pre-warmed growth medium. **Do not add Blasticidin, Puromycin and Zeocin™ until the cells have been passaged twice.**
4. Centrifuge vial at 1000-1200 RPM (RCF 200-300 g) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selection antibiotics.
6. Transfer the vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium without selection antibiotics.
7. Place the culture at 37°C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freezing medium freshly prepared with cold growth medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Prepare 1 ml aliquots of cells in cryogenic vials.
3. Place vials in a freezing container and store at -80°C overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell Maintenance

1. Maintain and subculture the cells in growth medium supplemented with 10 µg/ml of Blasticidin, 1 µg/ml of Puromycin and 100 µg/ml of Zeocin™.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: A subcultivation ratio of 1:2 to 1:8 is recommended. As a general guide

1:2 split should be 70-80% confluent in 1 day
1:4 split should be 70-80% confluent in 2 days
1:8 split should be 70-80% confluent in 4 days

Cell Handling Recommendations

To ensure the best results, use HEK-Blue™ IFN-λ cells with less than 20 passages.

REPORTER ASSAY

Day 1:

1. To prepare the HEK-Blue™ IFN-λ cell suspension, gently rinse cells with pre-warmed phosphate buffered saline (PBS), detach the cells in the presence of PBS by tapping the flask or by using a cell scraper, resuspend the cells in fresh, pre-warmed test medium, and prepare a cell suspension at ~280,000 cells/ml.

Notes:

- Some FBS may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these enzymes which are thermosensitive.

- The response of HEK-Blue™ IFN-λ cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ IFN-λ cells.

2. Add 20 µl of each test sample per well of a flat-bottom 96-well plate.
3. Add 20 µl of IFN-λ such as recombinant human IL-28A at 3 ng/ml (positive control) in one well.
4. Add 20 µl of type I or type II IFN such as recombinant human IFN-γ at 3 ng/ml (negative control) in one well.
5. Add 180 µl of the cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37°C in a CO_2 incubator for 20-24 h.

Day 2:

1. Prepare the QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
2. Add 20 µl of induced HEK-Blue™ IFN-λ cells supernatant per well of a flat-bottom 96-well plate.
3. Add 180 µl of resuspended QUANTI-Blue™ Solution per well.
4. Incubate the plate at 37°C incubator for 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
Anti-hIL-28A-IgG	Neutralizing antibody	mabg-hil28a-3
Anti-hIL-28B-IgG	Neutralizing antibody	mabg-hil28b-3
Anti-hIL-29-IgG	Neutralizing antibody	mabg-hil29a-3
Blasticidin	Selection antibiotic	ant-bl-05
HEK-Blue™ IFN-α/β	IFN-α/β reporter cells	hkb-ifnab
HEK-Blue™ IFN-γ	IFN-γ reporter cells	hkb-ifng
Puromycin	Selection antibiotic	ant-pr-1
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
Recombinant human IFN-γ	Recombinant cytokine	rcyec-hifng
Zeocin™	Selection antibiotic	ant-zn-1

QUANTI-Blue™ Solution

Medium for detection and quantification of alkaline phosphatase in standard and HTS assays

Catalog code: rep-qbs, rep-qbs2

http://www.invivogen.com/quanti-blue

For research use only

Version 18D13-MM

PRODUCT INFORMATION

Contents

QUANTI-Blue™ Solution is available in two pack sizes:

• **rep-qbs** containing 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer to prepare 500 ml of QUANTI-Blue™ Solution sufficient for 25 x 96-well plates (standard procedure) or 20 x 1536-well plates (HTS screening)

• **rep-qbs2** containing 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer to prepare 1 liter of QUANTI-Blue™ Solution sufficient for 50 x 96-well plates (standard procedure) or 40 x 1536-well plates (HTS screening)

Required Material (not provided)

• Sterile water

• Sterile screw cap tube, glass bottle or flask

Storage and Stability

• Store QB reagent and QB buffer at -20 °C. Product is stable for 1 year at -20 °C when properly stored.

• Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8 °C and for 2 months at -20 °C. Keep reconstituted QUANTI-Blue™ away from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

• Physicochemical characterization (including pH, solubility).

• Functional assays using alkaline phosphatase or SEAP-expressing reporter cells.

DESCRIPTION

QUANTI-Blue™ is a colorimetric enzyme assay developed to determine any alkaline phosphatase activity (AP) in a biological sample, such as supernatants of cell cultures. QUANTI-Blue™ Solution changes from pink to a purple-blue color in the presence of AP.

Secreted embryonic alkaline phosphatase (SEAP) is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a GPI-anchored protein. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters.

FEATURES AND ADVANTAGES

• **Requires small samples of cell supernatants** - 20 µl is sufficient.

• **No need to process samples** - Preparation of cell lysates or heating of samples is not required.

• **Determine secreted AP activity without disturbing cells** - The same cell cultures can be repeatedly sampled for kinetic studies.

• **Assay can be completed in 30 min** - Hands-on time no longer than 10 min. The enzymatic activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™ Solution.

• **Wide dynamic range allows to detect low and high levels of AP** - No need to perform multiple sample dilutions.

• **Highly sensitive for quantitative measurement** - Higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity.

• **Extremely simple to use** - 1) Prepare solution with water, 2) add sample to detection reagent, 3) incubate at 37 °C, and 4) assess AP activity.

METHODS

QUANTI-Blue™ Solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high throughput screening procedure).

A. Standard procedure

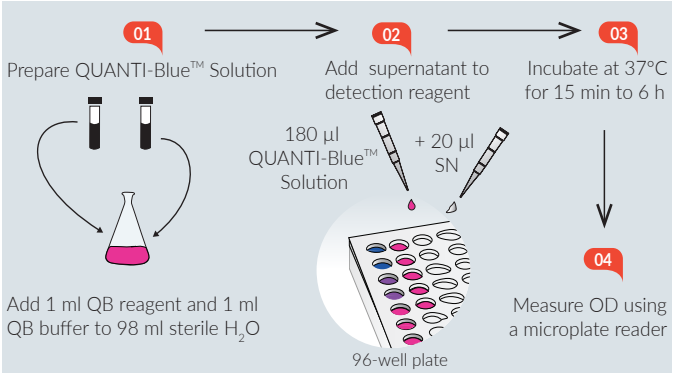


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

The following protocol refers to the use of 96-well plates. Ensure QB reagent and QB buffer are completely thawed before use.

*Note: For fast thawing, QB reagent and QB buffer can be placed at 37 °C for 2 minutes. Ensure heating at 37 °C does **not** exceed 5 minutes.*

1. Prepare 100 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water in a sterile glass bottle or flask.
2. Mix well by vortexing and incubate at room temperature for 10 min before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
4. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
6. Incubate at 37 °C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend to heat FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

For different cell culture plate formats, please refer to the table below:

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

B. High Throughput Screening procedure

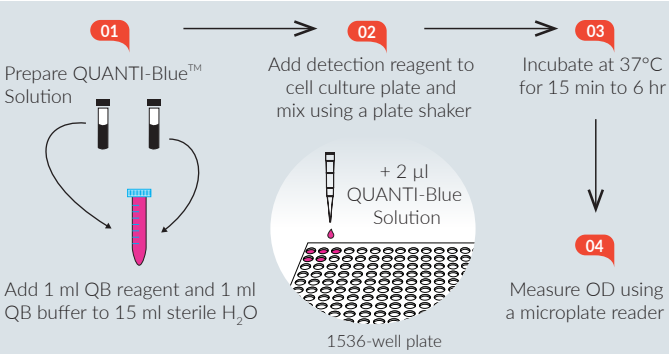


Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

This procedure has been optimized for use directly in flat-bottom 1536-well plates, in which cell culture volume does not exceed **5 µl**. Ensure QB reagent and QB buffer are completely thawed before use.
*Note: For fast thawing, QB reagent and QB buffer can be placed at 37 °C for 2 minutes. Ensure heating at 37 °C does **not** exceed 5 minutes.*

1. Prepare 17 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 15 ml of sterile water in a 50 ml screw cap tube.
2. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
4. Dispense 2 µl of QUANTI-Blue™ Solution per well of a 1536-well plate.
5. Mix using a plate shaker.
6. Incubate at 37 °C for 15 min to 6 h.
7. Measure OD at 620-655 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend to heat FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo^R)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
Reporter cells	
HEK-Blue™ hTLR2	hkb-htlr2
HEK-Blue™ hTLR4	hkb-htlr4
RAW-Blue™ Cells	raw-sp
THP1-Blue™ NF-κB Cells	thp-nfkb
THP1-Blue™ ISG Cells	thp-isg

For a complete list of InvivoGen's Reporter Cell Lines visit
http://www.invivogen.com/reporter-cells